Biomedicine & Pharmacotherapy 68 (2014) 911–916

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Review

Colon cancer: Cancer stem cells markers, drug resistance and treatment

Zuzana Kozovska [a,*,1], Veronika Gabrisova [b,1], Lucia Kucerova [a]

[a] Department of Oncology, Cancer Research Institute, Slovak Academy of Sciences, Vlarska 7, 83391 Bratislava, Slovakia
[b] Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Odbojarov 10, 83232 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 12 August 2014
Accepted 15 October 2014

Keywords:
Colon cancer
Cancer stem cells markers
Multidrug resistance
Aldehyd dehydrogenase ALDH1

ABSTRACT

Malignant tumours consist of heterogeneous populations of tumour cells. Cancer stem cells (CSC) represent a population of cells within a tumour with highly tumorigenic and chemoresistant properties. These cells may be identified by the expression of CSC markers. There are several key stem cells markers specified for colon cancer: CD133, CD44, ALDH1, ALCAM. These days, a major obstacle to effective cancer management is development of a multidrug resistance (MDR). The principal mechanism responsible for development of MDR phenotype is the over-expression of ABC transporters. Tumours and relapsing tumours after therapy are drived by subpopulations of tumour cells with aggressive phenotype resistant to chemotherapeutics. These cells are called CSC or tumour-initiating cells (TIC). Here we outline recent information about MDR of colon cancer and CSC markers. We have focused on novel therapeutic strategies which have been developed to prevent or overcome MDR. One such strategy is a combination of chemotherapy and modulators of MDR pumps or chemotherapy and monoclonal antibodies against vascular endothelial growth factor VEGF. Colon cancer is characterized by the presence of colon CSC expressing specific stem cell markers. The divergent presence of these markers can help to adjust personalized therapy. The review provides a detailed overview of resistance of colon cancer cells and discusses how the presence of CSC markers can influence therapy and prognosis of patients.

1. Introduction

Cancer is a genetic disease characterized by uncontrolled cell growth and the ability to invade other parts of the body by forming metastases. Malignant tumours consist of heterogeneous populations of cancer cells. CSC form a subpopulation of cells within a tumour which display the capacity of self-renewal and differentiation. These cells, also described as TIC, are presumed to promote growth and metastases of tumours [1].

There were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide (http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx). GLOBOCAN 2012 (IARC), Section of Cancer Information (16/6/2014).

The chemotherapy failure is attributed to the development of a MDR. The MDR phenotype accounts for unsatisfactory low response rate of solid tumours to chemotherapy in patients treated chronically with antineoplastic agents. This clinical phenomenon results from the ability of cancer cells to become simultaneously resistant to many structurally dissimilar and functionally divergent drugs used in anticancer therapy [2]. The over-expression of ABC transporters represents the principal mechanism by which cancer cells develop MDR. Increased drug efflux mediated by ABC transporters leads to decreased intracellular drug accumulation, hence to the decreased bioavailability. Combination therapy using more than one medication at the same time may be used to overcome MDR [3].

2. Progression of cancer

Several types of pre-malignant lesions, such as dysplasia and hyperplasia, can be detected in diverse organs prior to the appearance of fully malignant invasive tumours. An early stage of cancer characterised by the absence of invasion of tumour cells into the surrounding tissue, before penetration through the basement membrane, is called carcinoma *in situ*. Accumulation of genetic alterations occurs in one (or a few) of the pre-malignant cells, and the cells convert into malignant ones of clonal origin and produce a primary tumour. After the initial transformation and growth of cells, neoangiogenesis arises. At the early stage of

* Corresponding author. Tel.: +421 2 59327418; fax: +421 2 59327250.
E-mail address: zuzana.kozovska@savba.sk (Z. Kozovska).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biopha.2014.10.019
0753-3322/

primary tumour expansion, the cells are not invasive and metastatic. Invasiveness and metastatic ability appears as a result of further accumulation of genetic alterations in the cells. Diagnosis and treatment of cancer occurs generally late in the course of disease, in some types of cancer perhaps more than 10 years prior to the acquisition of the invasive phenotype [4]. At this stage, a high proportion of patients have obvious or occult metastases.

3. Metastases

Despite the significant improvements in diagnosis, surgical techniques, specificity of cancer treatment, patient care and adjuvant therapies, yet there is no cancer treatment that is able to cure majority of the patients with metastatic disease. The major obstacles to effective treatment include the biologic heterogeneity of tumour cells, the specific organ microenvironment, which influences the biologic behaviour of metastatic cells, including their response to systemic therapy and the development of drug resistance phenotype in metastatic cancer cells [5–7]. New and very promising approach for treatment of the metastases is a gene therapy using genetically modified mesenchymal stromal cells (MSC), which are able to convert a nontoxic prodrug to a highly toxic chemotherapeutic at the site of the tumour (so-called gene-directed enzyme/prodrug therapy; suicide gene therapy) [8–12].

4. Cancer stem cell theory

Cancer is known to result from the accumulation of multiple genetic mutations in a single target cell, sometimes over a period of many years [13]. Although monoclonal in origin, most tumours are recognised as a morphologically heterogeneous population of cancer cells [14]. This tumour heterogeneity can be explained by variations in tumour microenvironment and genomic instability [15–17]. CSC theory brings additional explanation, stating that intratumour heterogeneity can result from a functional diversity among cells in different states of differentiation [18]. Cancer cells within tumours may display different phenotypes, somewhat reminiscent of the normal tissue from which they originate, and have varying proliferation potential [19].

The CSC theory proposes that tumours have a cellular hierarchy that is a caricature of their normal tissue counterpart because they reflect the pluripotency of the originally transformed cell [20]. According to this hypothesis, only a subpopulation of cells within a cancer, made up of so-called CSC or TIC, have the exclusive capacity to regenerate a tumour and sustain its growth. Purified CSC are known to be potently tumourigenic [21]. They are able to regenerate the tumour from which they were derived when a limited number of cells (sometimes as few as 10^2 cells) are injected into an orthotopic in vivo microenvironment [20,22]. The other cancer cells have only a limited capacity to replicate and thus contribute to the tumour bulk but not to tumour maintenance [20].

CSC demonstrate some properties of normal stem cells, such as self-renewing abilities and differentiation capacity. Self-renewal is a unique replication process that allows stem cells to divide and give rise to either one or two stem cells [23]. In normal tissues, the expansion of the stem cell pool is restricted to prevent uncontrolled growth [24], whereas extensive and indefinite self-renewal is observed in CSC. CSC either originate from normal cells that became malignant or committed cells that acquired self-renewal capacity. Asymmetric division enables CSC to self-renew, thus forming another CSC, and a more committed progenitor cell. Subsequently, progenitor cell divides and differentiates to form a malignant progeny [25]. CSC are able to self-renew in vivo after serial transplantation in secondary and tertiary recipients when phenotypically identical and heterogeneous tumour is observed [21]. Differentiation is the second function of a stem cell and involves the potential to form tissue-specific specialized cells. Tumours arising from CSC demonstrate differentiation capacity in vivo by creating a phenocopy of the original human tumour.

4.1. Colorectal CSC

There is now accumulating evidence for the existence of colorectal CSC in human colorectal cancer (CRC) [18,26–29]. Using the CD133 surface marker in two separate studies [26,27], highly tumourigenic and self-renewing colorectal CSC population in human colon cancers have been successfully enriched and $CD133^+$ human cancer cells were found capable of inducing tumour formation while $CD133^-$ cancer cells failed to do so [27]. The $CD133^+$ cells produced passageable sphere-forming cells that remained undifferentiated for more than one year and were able to initiates in vivo tumours that were phenotypically similar to the original tumour [27]. In a renal capsule xenograft model, $CD133^+$ cells could generate tumours, but $CD133^-$ cells were unable to do so [26]. Moreover, $CD133^{high}$ populations displayed a much more aggressive phenotype than $CD133^{-/low}$ cells, in terms of metastases, implicating CD133 as a marker of increased tumourigenicity [30]. In addition, $CD133^+$ single cell clinical tumour-derived cultures were shown to possess multilineage differentiation potential and were capable of tumour initiation in vivo [31]. $CD133^+$ overexpressing tumours were more resistant to 5-FU-based chemotherapy and CD133 expression was associated with poor prognosis [32]. RNA interference approach showed that knockdown of CD133 had no effect on in vitro clonogenicity or in vivo xenograft tumour formation, demonstrating that CD133 is rather a passive marker of colorectal CSC [33]. Another subset of tumourigenic human colon cancer cells was identified based on $ESAhighCD44^+$ $CD166^+Lineage-$ colon cancer phenotype [18]. Knockdown of CD44 (hyaluronate receptor, P-gp 1) resulted in limited colony formation and dramatically reduced tumour formation in xenografts, strongly implying a functional role of CD44 in CRC tumourigenesis [33].

Aldehyde dehydrogenase 1 (ALDH1) is a promising new marker for identification of colorectal CSC. ALDH was found to be a specific marker for identification, isolation and tracking of malignant human colonic CSC and for quantifying the number of CSC over the course of CRC development [28]. Flow cytometric isolation of cancer cells based on enzymatic activity of ALDH (ALDEFLUOR-assay) and implantation of these cells in NOD-SCID mice:

- generated xenograft tumours ($Aldefluor^-$ cells did not);
- generated them after implanting as few as 25 cells, and;
- generated them dose dependently [28].

ALDH is an intracellular enzyme that oxidizes aldehydes, possess a detoxifying role, converts retinol to retinoic acid [34], mediates control over differentiation pathways [35], plays an important role in self-protection of normal stem cells [36] and confers resistance to alkylating agents. ALDH is now widely used as a marker for identification and isolation of various types of normal stem cells and CSC. ALDH can confer resistance to selected anticancer agents by metabolic inactivation [37] and it is speculated to be a cause of relapse in cancer patients [38–40]. ALDH1A1 is one of the 19 ALDH isoforms expressed in humans. In many cancers, high ALDH1 expression is associated with metastases development and correlates with poor clinical outcome [41,42]. Recent evidence suggests that ALDH activity may protect against cell death caused by reactive oxygen species (ROS) [43]. ALDH activity can be inhibited pharmacologically by